THE EMULSIFYING PROPERTIES OF MEAT PROTEINS[a]

Clifton E. Swift
Eastern Regional Research Laboratory[b]
Philadelphia, Pennsylvania

A thoroughly considered definition of an emulsion has been proposed by Becher (1) as follows: "An emulsion is a heterogeneous system, consisting of at least one immiscible liquid intimately dispersed in another in the form of droplets, whose diameter, in general, exceeds 0.1u." Such systems possess a minimal stability, which may be accentuated by additives such as surface-active agents. An oil/water emulsion consists of a disperse phase (the droplets of oil) and a continuous phase (the water). A stabilizer acts at the interfaces between the disperse and continuous phases. The activity of meat proteins at the surface of fat in emulsion based meat products is the special case that concerns us.

While emulsion theory and practice have provided a good deal of information on the mechanisms of emulsion stabilization, emulsification with proteins as stabilizers has been investigated to only a limited extent. Review of the literature indicates that interest has principally been in learning the behavior of peptides and proteins at interfaces as a means of elucidating the structure of proteins and the mechanisms of biochemical processes, with little direct interest in such emulsions as those made in meat processing. In any case, characterizing the events involved in making a meat emulsion is quite complex and will require more investigation than has been undertaken to date. However, the information presently available on the behavior of peptides and proteins at interfaces and some of the still developing knowledge of the chemical and physical characteristics of muscle proteins furnishes a basis for considering the general nature of the mechanisms involved.

The behavior of proteins at oil/water interfaces can be expected to vary, depending on the chemical and physical characteristics of these proteins, including even the most subtle aspects of their structure. Proteins are macromolecules formed

---

[a]Reprinted from PROCEEDINGS OF THE MEAT INDUSTRY RESEARCH CONFERENCE. Sponsored by American Meat Science Association in Cooperation with American Meat Institute Foundation at Center for Continuing Education, University of Chicago, Mar. 25, 26, 1965. Litho. in USA.

[b]Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

by a large number of amino acids (Figure 1,a) condensed by removing the elements of water from amino and carboxyl groups to form a chain joined by the peptide linkages that elimination of the elements of water produces (Figure 1,b). From 20 different amino acids, arranged in different number (up to thousands) and sequence, obviously an extremely large number of proteins can be formed. Along the peptide backbone of the structure, differences in the amino acids are reflected in different substituents on -carbons, i.e., R_1, R_2, R_3, etc. Differences in the behavior of these groups, when exposed to water and to oil and fat, account, in part, for the manner in which the protein molecule interacts at the oil/water interface. Non-polar groups are attracted to the oil phase, while such groups as amino, imino, amide, imidazole and carboxylic groups are attracted to the water phase. They may be available at the interface and available for interaction; but, on the other hand, they may be buried in the structure of the protein.

This fact directs attention to the importance that three dimensional structure can have in determining how proteins will behave at interfaces and to variations in structure. For a given protein this structure should correctly reflect the net effects of C to C, C to N, and other interatomic bonds and their correct angles, hydrogen bonding, disulfide bonding, salt bridges, and the attraction of non-polar groups for each other, which give proteins their characteristic shapes. The structure of part, or even most, of some proteins is described by the -helix, the form of which was postulated in 1951 by Pauling, Corey and Branson (2). The construction of a peptide in -helix form is shown in Figure 2 Shown protruding are -carbon substituents, shaded here for easy observation, which will interact at the oil/water interface, depending on their nature, as was previously discussed.

However, proteins frequently are found to assume the -helical form, in part, and, in other parts, the random coil. This coiling tends to produce a protein which is relatively compact and, with appropriate cross-linking and hydrogen bonding, can produce a molecule which is not only compact but relatively rigid. Myoglobin is an excellent example of this type of protein. In it 65-72% of the structure is -helical and the rest non-helical. The outstanding work which determined this structure was that of Kendrew et. al. (3). A drawing showing the direction of coils in myoglobin is presented in Figure 3; in it the straight -helical sections and also coiled, non-helical sections may readily be distinguished.

If we consider the structure of meat proteins, which includes myoglobin, of course, we find that our knowledge is far from complete, although a great deal of investigation has been and is being done. Table 1 contains, in summary fashion,

PROTEIN COMPOSITION AND PRIMARY STRUCTURE

(a) $(RCH(NH_2)COOH)_n$

(b)

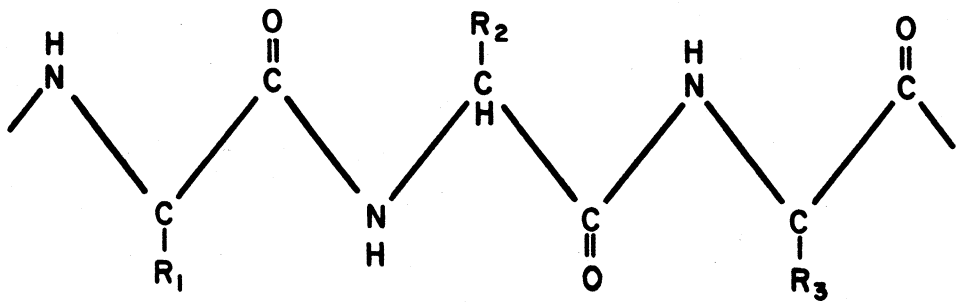

Figure 1. (a) General amino acid structure where the amino acid occupies a position on the carbon alpha to the carboxyl group and where side chain R may be of diverse composition.
(b) The polypeptide backbone.

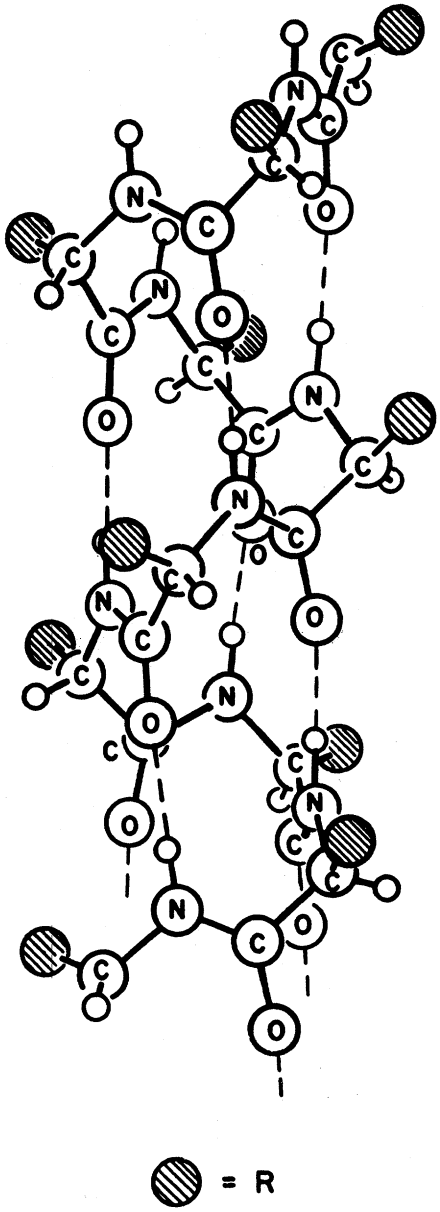

Figure 2. A sketch of a section of a polypeptide having α-helical structure.

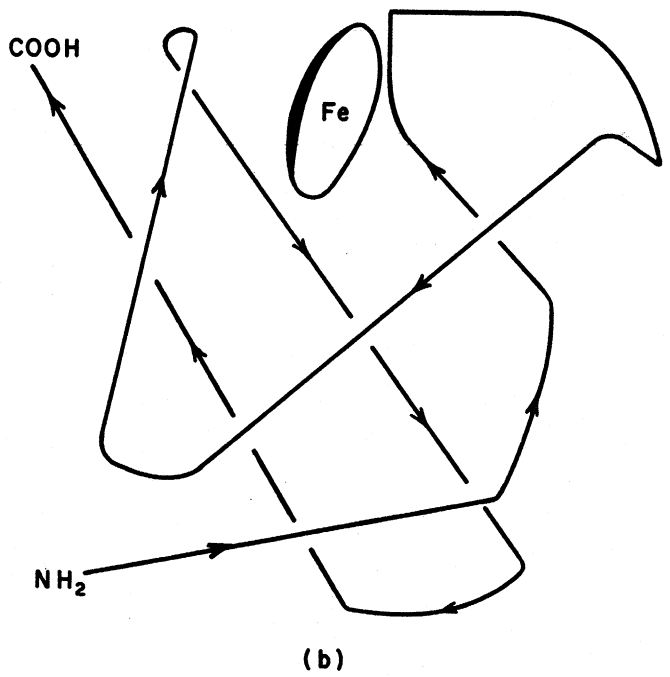

Figure 3. A sketch in which the direction of coiling in the myoglobin molecule is shown where straight sections are α-helical and others are randomly coiled.

much of the more recent conclusions on the structure of G- and F-actin, myosin, actomyosin, and tropomyosin, largely selected from a review published in 1964 by Kielley (4). These

Table 1. Characteristics of Some Muscle Proteins

Protein	Molecular weight	Structure
G-Actin	60,000	Ellipsoid
F-Actin	--	Two wound strands of spheres
Myosin	600,000	Three wound α-helical strands
Actomyosin	3:1-4:1 myosin to actin	--
Tropomyosin	54,000 (monomer)	α-helical

proteins make up approximately 80% of the myofibrillar proteins. The G-actin molecule appears to be generally spherical, presumably indicative of coiling. F-actin appears to be a double stranded, linear array of spherical subunits; and, while it is fibrous in overall nature, its subunits may be described as spherical like those of G-actin. Myosin structure for the most part is α-helical; in fact, it consists of three strands of α-helices, but, in addition, it consists of a globular section at the end of the molecule. Tropomyosin has an α-helical form which is actually double helical. Actomyosin structure is not described but as a product of myosin-actin association it can be expected to have a high α-helical content. Assuming that the so-called water-soluble meat proteins are globular and, thus, randomly coiled compact moleucles, appears reasonably based on general concepts. This, of course, is a generalization, taking no account for the variety among this group which comprises approximately 30% of the total protein in muscle tissue.

Obviously, owing first to variation in the surface activity of their individual complement of amino acids and second to differences in structure, these different meat proteins should exhibit different behavior at oil/water interfaces. A complete accurate knowledge of this behavior must await further detailed investigations, but, even now, the literature provides some insight into the probable nature of the interactions involved. A quite extensive review of knowledge of this subject has been presented by Cheesman and Davis (5) and a very pertinent, more recent, discussion of monomolecular layers presented by

Katchalski, et. al. (6). Evidence points to an unfolding, or at least, a partial unfolding of peptides and proteins at interfaces, where there is a tendency to form monomolecular layers of peptide chains. The evidence points to a release of forces which permits not only an unfolding but an orientation, or twisting, of peptide chains in order that the various functional groups of side chains, depending on their hydrophobic or hydrophilic nature, can best be accommodated in the oil or water phases to which they are attracted. Apparently the original proteins become denatured. However, as a note of caution against generalization, it should be recognized that many proteins apparently maintain serological specificity and biological activity in interfacial films, as has been pointed out by Kaplan and Fraser (7). In fact, it appears that enzymes in some cellular systems may normally be situated and active as films, or membranes, and, certainly, in a most important sense, are not denatured. It can be expected, then, that emulsification produces modifications in meat proteins of varying severity, which affects biochemical activity, perhaps drastically, although not in any presently predictable manner.

The emulsifying capacity and the emulsifying ability of meats, meat proteins and extracts have been studied in some detail in recent years. In our laboratory it followed water-binding studies to which it is related. Determining the maximum amount of fat that a given amount of meat or protein can convert to an emulsion, at least momentarily stable, is a measure of capacity. On the other hand, determining the stability of an emulsion formed with a meat or protein under conditions not necessarily approaching maximum utilization of the stabilizer is a measurement of a different quality. In our work and that of some others the distinction has sometimes been overlooked. The results of Hegarty, et. al. (8) indicate that certain important differences may exist, as will be noted later.

In the investigations of several groups of workers a method has been employed which is designed to measure emulsifying capacity, i.e., a determination in which all the fat possible is emulsified, under stated conditions, by a given amount of meat slurry, extract, or protein. This is the type of method applied in our laboratory (9), by Hegarty, Bratzler and Pearson at Michigan State University (8), and by Carpenter and Saffle at the University of Georgia (10). With this method, liquid fat is added to a stirred aqueous solution or suspension of meat slurry, extract, or protein until an emulsion forms and then breaks. A simple arrangement of equipment is shown in Figure 4; however, it should be noted that the apparatus has subsequently been modified so that the fat is maintained at constant temperature (ca. 5°) by cold baths until it is combined with

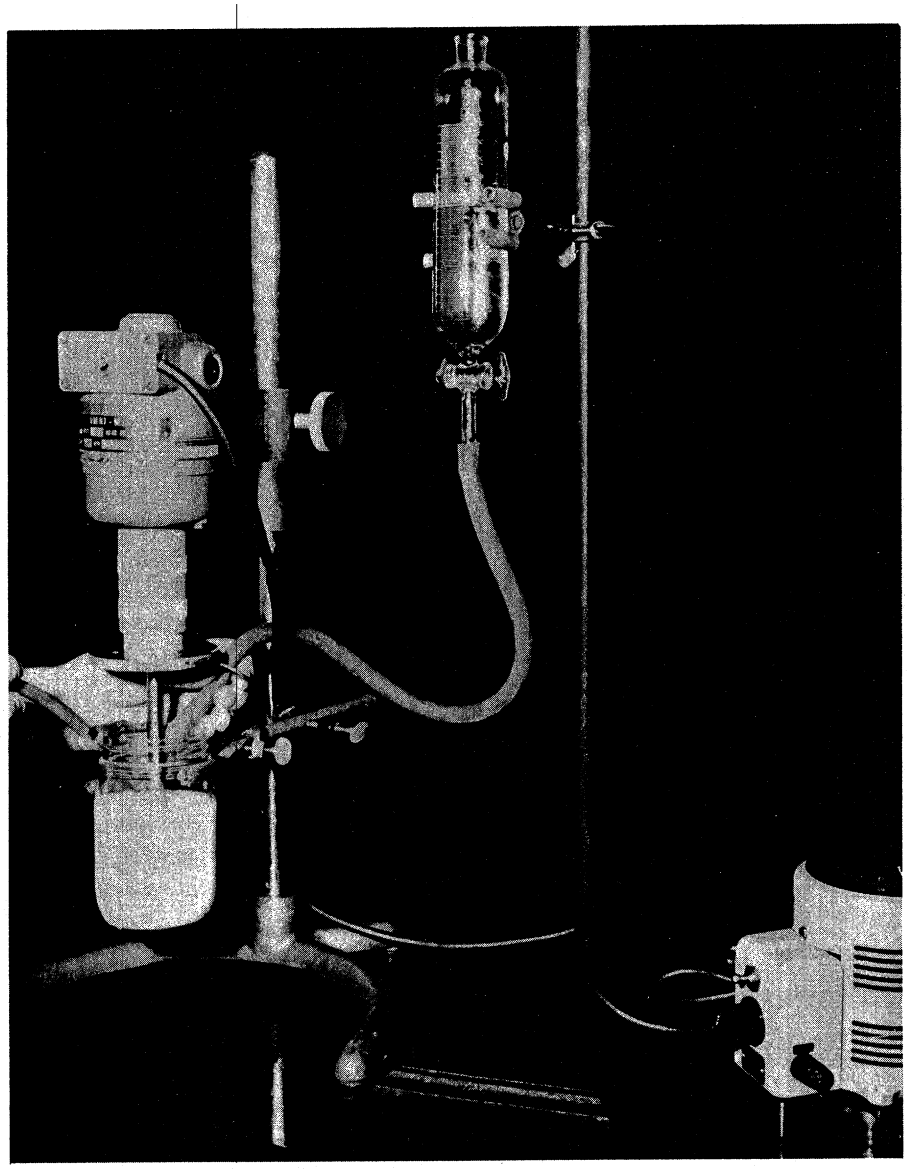

Figure 4. Apparatus arranged for determinations of emulsifying capacity.

other components of the emulsion.

Related methods can be used to determine the stability of an emulsion by adding somewhat less fat than can be emulsified at the capacity level and observing its stability during storage. This method has been applied by Hegarty et. al. and by Trautman of Oscar Mayer (11).

As listed in Table 2, a number of factors affect emulsification with meat proteins as stabilizers including: (1) the initial comminution of tissue, prior to emulsification, in order to free and solubilize proteins; (2) the rate of mixing during emulsification; (3) the rate of addition of fat; (4) the temperature, (5) pH; and, (6) salt concentration (9, 14). These factors are of interest not only as factors affecting laboratory determinations but, also, because the effects relate, to some extent, to emulsification in practical sausagemaking.

Table 2. Variables Influencing Emulsification.

Variable	Oil emulsified as variable increases
Mixing	Reduced
Temperature	Reduced linearly
Concentration protein	Reduced efficiency
pH	Increased
Salt concentration	Increased

The objective in comminution is to make the proteins available and if not to dissolve them then to break them away from structural tissue. The parameters usually important in extracting proteins from tissue appear to apply. The operation must be accomplished at low temperature, fine particle size must be obtained and, at the same time, excessive comminution which tends to denature proteins must be avoided.

Emulsification is influenced by variations in the rate of mixing of H_2O-oil phases, since mixing rates directly affect the degree to which fat becomes dispersed. Increasing the rate of mixing, or shearing force, decreases fat globule size and increases surface area, and thus a larger area of protein membrane becomes necessary at interfaces. It is logical to assume that smaller globules with their appropriate membrane are physically strongest, having most structural support from membrane per unit volume of oil or fat.

Temperature influences emulsifying capacity, low temperatures markedly favoring increased emulsifying capacity.

Actually the cause of the "temperature effect" is unresolved. The factors possibly accounting for it are that: (1) warm, more limpid fat can be more highly dispersed and thus will require more protein to coat surfaces; (2) the protein may become partially denatured and thus less effective as temperature increases; and, (3) membrane formation may somehow differ at higher temperatures.

The effect of varying rates of adding fat to protein suspensions is interesting. According to Ascherson's observation in 1840, membrane formation appeared instantaneous when egg protein and olive oil were allowed to come into contact (12), as, indeed, it appears in emulsifying with meat proteins. Results from our laboratory and those of Carpenter and Saffle (10) are not in agreement, since only ours show that rapid addition of fat increases emulsifying capacity. The operation of emulsification in our laboratory procedure requires 2-3 minutes, but could be accomplished more rapidly if detection of an "end point" was not involved. Apparently, slowing the addition of fat in a potentially rapid reaction, in which formation and physical destruction of emulsion obviously occur simultaneously, reduces the rate of emulsion formation while allowing unnecessary physical destruction of the emulsion to occur. The result is less fat emulsified per unit of protein. It probably has its counterpart in over-chopping in sausagemaking.

Less fat is emulsified per unit of protein as protein concentration is increased in the laboratory procedure. We observed this in our laboratory, while Hegarty et. al. (8) observed a curvilinear relation between the concentration and emulsifying capacity of several proteins studied. While dilution appears to increase emulsifying efficiency, it is a possibility that multilayer membranes may be formed from more concentrated protein extracts and the membranes could be stronger. On the basis of the evidence, however, the data lead to a prediction that emulsification in ordinary sausagemaking will be relatively inefficient, which it decidedly is by comparison with laboratory tests using dilute protein extracts.

pH affects emulsification through its marked influence on the properties of proteins. A list of pH effects on proteins includes those on solubility, ozmotic pressure, light absorption, viscosity, electrophoretic mobility, stability, enzymic activity, binding of other ions, and sometimes molecular weight, as enumerated by Steinhardt and Beychok (13). In emulsification with meat proteins, pH values higher than 5.4 are needed to achieve pH values above the isoelectric point (pH of minimum solubility) of myosin and actomyosin to promote solubilization. A pH of 6 or above is advisable, according to results obtained in our laboratory. The results of Hegarty et. al. showed that pH

values of approximately 5.5 yielded the best emulsions with sarcoplasmic proteins but that pH values near neutrality gave better results with myosin and actomyosin. Observations in our laboratory showed that water-soluble proteins have maximum activity at about pH 5.25; however, it is not practical to sacrifice the emulsifying capacity of salt-soluble proteins in order that this relatively low value be obtained in emulsifying with mixtures consisting of both water- and salt-soluble proteins.

Finally, salt concentration has an influence (8, 14). The most obvious reason is that an ionic strength of 0.5, or higher, is needed to extract salt-soluble proteins from tissue. Moreover, the water-soluble proteins are increasingly effective as stabilizers as salt level increases. This improvement appears to be the result of changes in the stability of the structures of some of the proteins which promotes their tendency to unfold and spread as a monomolecular film. Regarding the effect of both pH and salt concentration on emulsifying capacity, attention should be directed to a recent investigation of the relation of changes in charge, shape and viscosity of meat proteins as related to emulsifying capacity conducted by Carpenter and Saffle (15). In brief, their conclusions are that measurements of emulsifying capacity reflect changes in both charge and shape of the proteins involved. As indicated earlier, meat proteins differ widely in chemical composition and structure. If their ability to form membranes at interfaces reflects attraction to either fat or water phases, depending on differences in amino acids, and reflects molecular structure, which determines tendencies towards unfolding and deformation, it is only logical to assume that the proteins will produce emulsions in different amounts and stability. Some of the evidence obtained in studies of protein films indicates that there can be a partial unfolding at interfaces. If some proteins have this tendency and not others, membranes would differ in structure and, very likely, in strength. In any case, as will be evident in results to be discussed, there appears to be differences in the stability of emulsions formed with different meat proteins. Fortunately, information is available on both the quality, as well as the quantity of emulsification that can be obtained with meat proteins, although further need for investigation undoubtedly exists.

In brief outline, some of the results of investigations of the relative emulsifying capacity and emulsion stability obtained with extracts and proteins are shown in Table 3. In work published by Trautman (11), a comparative test of the stability of prerigor and postrigor salt-soluble pork proteins was made. The results showed that prerigor proteins stabilized emulsions for considerably longer periods, amounting to several times as long, by his method. Probably the same would be true of beef proteins. The explanation advanced was that there were differences in the

percentage of salt-soluble proteins extracted, 43% as against 39%, which is not too large a difference, and the "condition" of the proteins. Undoubtedly the term "condition" was used in a fully enlightened sense, for the observed difference invites postulation that the prerigor protein, myosin, had greater effectiveness than postrigor actomyosin. However, the results of Hegarty et. al. (8) show these two proteins to be comparable. It is therefore advisable to concur in the use of the term "condition" admitting that too little is known about the changes accompanying rigor to explain the observed superiority of prerigor proteins.

Table 3. Relative Stabilization by Extracts and Proteins

Characteristics	Superior stabilizer
Stability	Prerigor salt soluble proteins
Capacity and stability	Salt-soluble protein
Capacity	Actin > myosin > actomyosin > sarcoplasmic proteins
Stability	Myosin, actomyosin and sarcoplasmic proteins

All of the evidence available regarding the comparative emulsifying capacity of salt-soluble proteins versus water soluble proteins indicates that salt-soluble proteins are definitely superior. The question is how much so.

According to the results obtained in our laboratory, salt-soluble proteins were from around 30% to 400% more effective, even when the water-soluble proteins were employed under favorable circumstances. Prior to this, Hansen (16) had reported that water-soluble proteins were ineffective; however, the effect of salt was not investigated. More recently, Trautman also reported that water-soluble proteins had relatively little emulsion stabilizing capacity (11). In these tests, emulsion stability rather than formation was measured. Perhaps due to differences in the method used, 300 mg. of protein failed to stabilize only 5 ml. of fat, whereas many times that much fat would apparently have been emulsified using the method employed in our laboratory or that of Hegarty et. al. The results of Hegarty showed that water-soluble sarcoplasmic proteins have emulsifying capacity both in only water, or in water plus salt; more oil per unit of protein was emulsified without salt.

Obviously, the relative importance of the water-soluble proteins is not firmly established in view of the varying degrees of disagreement among the results reported. However, the evidence

that these proteins are effective, although not so effective as salt-soluble proteins, is the more convincing. If the sarcoplasmic proteins do indeed have a moderate ability to emulsify fats, their practical importance can be appreciable. The obvious reason is that since water-soluble proteins need no solubilization or release from the tissues to be effective, they are completely available.

The results obtained by Hegarty et. al. with actin are especially interesting. The fact that actin in salt solution had a poor emulsifying capacity and stability is only mildly interesting since there is doubt that much free actin exists in either prerigor or postrigor meat. However, the wide difference between the capacity of actin to form an emulsion (in the absence of salt) and the stability of the emulsion is of decided interest. This clearly suggests that estimates of both capacity and stability are required in applying emulsification measurements for the evaluation of meats. In fact it appears advisable to go even further and to routinely determine the stability of heated emulsions as a means of obtaining the most realistic guidance for practical sausagemaking.

Data available on the emulsifying capacity of different meats is limited to the work of Carpenter and Saffle (10), although considerable unpublished work has probably been done in industrial laboratories. Part of their results were used in calculating the amount of oil emulsified by 100 gm. of meat for several different meats as shown in Table 4. It is readily apparent that the relative order of the meats corresponds with the reported value of meats in sausagemaking.

Table 4. Emulsifying Capacity of Meats

Type	Oil/100 gm., ml.
Bullmeat	3100
Boneless cow	3000
Beef cheek	2280
Lean pork trimmings	1920
Beef tongue	1580
Beef heart	1500
Tripe, jowls	<600

It appears that emulsification tests, conducted with regard to both capacity and stability, even in the developing stage, are now more accurate than any alternative. If the protein content of extracts are used for comparisons of meats, as has been proposed, it is obvious that estimates of only salt-extractable proteins fail to distinguish between the value of prerigor and postrigor proteins, or take account of the contribution of water-soluble proteins.

Determining total soluble proteins fails because it equates water-soluble proteins with the superior salt-soluble proteins. Moreover, there is no present justification for equating proteins from different tissues, whether they are different muscles, from different animals, or from different species. For practical purposes, fat emulsification tests appear to obviate the need for considering the complexities and variants that exist in meat materials and for research purposes afford a tool for investigating these complexities and variants.

Considering the foregoing it is readily apparent that much remains to be learned about the emulsification process. The following comprises a partial list of needs:

1. Refinement of emulsification test methods.
2. Resolution of differences that exist between observations of different groups of workers.
3. Characterization of the detailed interactions between proteins and fat interfaces. Do proteins, or emulsifiers, having low emulsifying capacity decrease the effectiveness of those that are effective? The possibility is suggested by work of Meyer et. al. (17) which shows that the addition of some commonly accepted emulsifiers to sausage decreased rather than improved emulsification.
4. Extension of knowledge of the meat proteins themselves and their behavior.
5. Extension of knowledge of meat composition.
6. Measurement of the differences attributable to different types of fat.
7. Adaption of the newly developing rational of the emulsification process to practical sausagemaking.

Literature Cited

1. Becher, Paul. 1957. "Emulsions: Theory and Practice." Reinhold Publishing Corp., N. Y.

2. Pauling, L., R. B. Corey and H. R. Branson. 1951. The structure of proteins: two hydrogen-bonded helical configurations of the polypeptide chain. Proc. Natl. Acad. Sci. U. S. 37:205.

3. Kendrew, J. C., R. E. Dickerson, B. E. Strandberg, R. G. Hart, D. R. Davies, D. C. Phillips and V. C. Shore. 1960. Structure of myoglobin. Nature. 185:422.

4. Kielly, W. W. 1964. The biochemistry of muscle. Ann. Rev. Biochem. 33:403.

5. Cheesman, D. F. and J. T. Davies. 1954. Physico-chemical and biological aspects of proteins at interfaces. Adv. Prot. Chem. 9:439.

6. Katchalski, E., M. Sela, H. I. Silman and A. Berger. 1964. Polyamino acids as protein models. V. Monomolecular layers. In: "The Proteins" (H. Neurath, ed.), Vol. 2, 2nd Ed., Academic Press, New York. 513.

7. Kaplan, J. G. and M. J. Fraser. 1954. The expansion of monomolecular films of ovalbumin. J. Biol. Chem. 210:57.

8. Hegarty, G. R., L. J. Bratzler and A. M. Pearson. 1963. Studies on the emulsifying properties of some intracellular beef muscle proteins. J. Food Sci. 28:663.

9. Swift, C. E., C. Lockett and A. J. Fryar. 1961. Comminuted meat emulsions - The capacity of meats for emulsifying fat. Food Tech. 15:468.

10. Carpenter, J. A. and R. L. Saffle. 1964. A simple method of estimating the emulsifying capacity of various sausage meats. J. Food Sci. 29:744.

11. Trautman, J. C. 1964. Fat-emulsifying properties of prerigor and postrigor pork proteins. Food Tech. 18:1065.

12. Ascherson, F. M. 1840. On the physiological utility of the fats and a new theory of cell formation based on their cooperation and supported by several new facts. Arch. Anat. Physiol. Lpz., p. 44; reprinted in Hatschek, E., "The Foundations of Colloid Chemistry." Macmillan Co., New York. 1925.

13. Steinhardt, J. and S. Beychok. 1964. Interaction of proteins with hydrogen ions and other small ions and moleucles. In: "The Proteins" (H. Neurath, ed.), Vol. 2, 2nd Ed., Academic Press, New York. 139.

14. Swift, C. E. and W. L. Sulzbacher. 1963. Comminuted meat emulsions: Factors affecting meat proteins as emulsion stabilizers. Food Tech. 17:224.

15. Carpenter, J. A. and R. L. Saffle. In press. Some physical and chemical factors affecting the emulsifying capacity of meat protein extracts. Food Tech.

16. Hansen, L. J. 1960. Emulsion formation in finely comminuted sausage. Food Tech. 14:565.

17. Meyer, J. A., W. L. Brown, N. E. Giltner and J. R. Guinn. 1964. Effect of emulsifiers on the stability of sausage emulsion. Food Tech. 18:1796.